LEE METCALF NATIONAL WILDLIFE REFUGE

BULLFROG AND PAINTED TURTLE INVESTIGATIONS: 1997

A Report to:

U.S. Fish and Wildlife Service

Lee Metcalf National Wildlife Refuge
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ABSTRACT

Studies of bullfrog and painted turtle populations on Lee Metcalf National Wildlife Refuge, Ravalli County, Montana, were conducted during summer 1997. Objectives were to determine the distribution of each species on the refuge and identify factors, such as the presence of bullfrogs, that might be affecting painted turtle recruitment. Both species are found in most water bodies on the refuge. Few sites were identified where bullfrog reproduction is occurring, but this probably resulted from inadequate sampling methodology. Hatchling and juvenile painted turtles were observed at several sites, indicating recruitment continues to occur, although it is not known if the rate is sufficient to maintain a viable population. Neither bullfrog (n = 21) nor largemouth bass (n = 37) stomachs contained hatchling painted turtles. Frequency of predation on turtles by these two species is probably low. Painted turtle nesting occurs across the refuge, but nesting sites appear more abundant in the north half of the refuge. Many depredated nests were found; fate of 8 nests found at the time of egg laying was 6 (75%) lost to predators and 2 (25%) undisturbed after 75-78 days of exposure. Rate of turtle nest loss on the Refuge is high relative to other studies, but our small sample may have resulted in a biased value. Potential predators included striped skunk, raccoon, coyote and possibly common raven.

INTRODUCTION

The bullfrog (*Rana catesbeiana*), an amphibian species native to the eastern and central U.S., was introduced to western Montana sometime before the 1960's (Black 1969) and today occurs in the Flathead and Clark Fork River drainages, including the Bitterroot Valley. They are widespread in the Bitterroot Valley and abundant on Lee Metcalf National Wildlife Refuge in Ravalli County. Introduced bullfrogs can threaten native amphibians through displacement by tadpoles and adults, and adult diet may include tadpoles of their own and other species of frogs. In their natural range bullfrogs also occasionally eat hatchling turtles and small snakes (Korschgen and Moyle 1955, Korschgen and Baskett 1963, McKamie and Heidt 1974). The effects of bullfrogs on the native amphibian and reptile species of the Refuge are not known.

The painted turtle (*Chrysemys picta*) is common and widespread across North America. This species occurs throughout Lee Metcalf NWR, and is the most visible reptile of the region; the apparent absence of hatchling turtles led refuge managers to express concerns of low population recruitment. Nest predation rates from mammalian carnivores appear to be high on the Refuge, a fate typical for turtle nests elsewhere (e.g., Tinkle et al. 1981, Snow 1982, Christens and Bider 1987, Temple 1987). Once eggs hatch, additional mortality to hatchling turtles could occur from the large introduced populations of bullfrogs and large-mouth bass (*Micropterus salmoides*).

In 1996, the Montana Natural Heritage Program initiated studies to determine the composition and status of refuge amphibians and reptiles (Hendricks 1997). Late summer surveys for bullfrogs, painted turtles, and other amphibians and reptiles were conducted. Turtles and bullfrogs were found to occur throughout the refuge, but no native frogs were observed. Because

work was initiated late in the year, surveys of greater duration and intensity were considered necessary to make recommendations regarding bullfrog and turtle populations on the Refuge.

Objectives for the 1997 field season were 1) conduct additional surveys of amphibians and reptiles on the Refuge, noting bullfrog and painted turtle presence in additional to general searches for other species, 2) locate painted turtle nests and follow their fates, 3) determine the food habits of adult bullfrogs on the Refuge, and 4) determine the food habits of largemouth bass on the Refuge. Some effort was also made to observe turtle basking sites to determine if a range of size classes use these sites; the presence of small turtles would indicate some undetermined rate of recruitment continues to occur on the Refuge.

METHODS

We attempted to conduct complete surveys of the waters of the Refuge during two time periods: 1) early in the summer (May-July), when water was high, and 2) late in the summer (August-September) when comparable work was conducted in 1996. We were interested in determining the occurrence and distribution of native amphibians and reptiles over time on the Refuge, and contrasting those results with the distribution of bullfrogs. We hypothesized that use of ephemeral ponds in early summer may favor native amphibians over exotic bullfrogs. Bullfrogs use aquatic habitats other than those found in ephemeral pools; bullfrog tadpoles, which must overwinter once before transforming to adults (Nussbaum et al. 1983), probably cannot survive in ephemeral ponds. Furthermore, bullfrogs emerge from hibernation in late spring, and their activity may not overlap with breeding activity of some native amphibians, which breed earlier in the spring.

Amphibian-reptile surveys were conducted over most of the temporary and permanent waters on the refuge, and in terrestrial habitats in wooded areas. Surveys were conducted by walking margins of ponds and puddles, and by floating large ponds with a small boat. Terrestrial searches were conducted in likely habitat throughout the Refuge, by turning over logs and searching under loose bark. Time of day, ambient temperature, and water temperature were recorded at the beginning and end of searches. The presence of each species was noted, and the number of individuals was counted directly or estimated (if individuals were vocalizing).

Size structure of the Refuge painted turtle population was investigated at focal basking sites. Size of turtles (carapace length: small [~10 cm], medium [~15 cm], or large [~20cm]) was based on visual estimates of turtles basking on logs of known length. In order to evaluate the status of painted turtle populations, we augmented general surveys of their occurrence across the Refuge with nest searches. We identified potential nest sites through discussions with refuge personnel and evidence of prior nest predation (excavations with scattered eggshell fragments). We found active turtle nests by locating females laying eggs at dusk. Turtle nests were marked with flagging, their precise locations mapped, and the approximate distance from each nest to water was noted. We followed the fate of nests by periodical nest checks. We also counted and mapped locations of all predated nests. We attempted to identify nest predators whenever possible from tracks and other evidence. We took photographs and collected eggshells of a representative sample of predated nests for verification of predators.

We collected bullfrogs to determined their food habits by spotlighting at night and capture with a dipnet. Bullfrogs were collected from Ponds 10, 13, and North Slough. Most frogs (n = 21) were collected early in the summer, but two were collected during late summer. The entire

GI tract was removed, fixed in formalin, and stored in ethyl alcohol. All items in the stomach were identified to the most precise taxonomic level possible. Estimates of the number of prey individuals in each sample were made from counts of body parts. Diet was quantified as 1) the number of prey individuals in each prey category relative to the total sample of items from all stomachs and 2) the proportion (expressed as a percentage) of stomachs in which a prey taxon occurred. We preserved a few bullfrogs as voucher specimens for the vertebrate museum at the University of Montana, Missoula. We also preserved the right front leg of each bullfrogs for potential aging using the growth rings on bones (this has not yet been done).

We examined food habits of largemouth bass in ponds where they are abundant (Ponds 8 and 10), to determine if hatchling turtles are part of the diet. We collected bass (n = 37) by fishing with hook and line, removed the GI tracts and preserved them as described for bullfrogs. Stomach contents were examined for turtle hatchlings, and all contents were identified (but not quantified) to the most precise taxonomic level possible.

RESULTS AND DISCUSSION

Bullfrog Surveys--Surveys were conducted early (21 June-30 July) and late (20 August-11 September) during summer 1997. Bullfrogs occurred throughout the refuge, and were more abundant in the northern portions of the refuge (Figure 1). They were locally abundant in Ponds 13, 12, 11, North Slough, Pond 10, and Pond 8. Other sites where bullfrogs were detected included Otter Pond, the Gravel Pit, a small slough between the Gravel Pit and Pond 10, two smaller ponds within the bounds of Mailbox Pond, Barn Slough, Ponds 5 and 6, and Ponds 1-3. We failed to detect bullfrogs in Pond 4 and Francios Slough. We counted 23 bullfrogs during a

Figure 1. Locations of bullfrogs on Lee Metcalf NWR during summer 1997. (unavailable)

nighttime search of North Slough. A subsequent daytime count around part of the Slough=s margin resulted in about twice as many frogs (44). If the total nighttime count is multiplied by 1.9 (the proportionate increase in numbers counted during the day relative to night), then we estimate we would have counted 82 frogs during a complete daytime count. This contrasted sharply with the 449 frogs detected during the daytime on 29 August 1996. The 1996 count occurred only about one week earlier than our 1997 count and is not likely to account for the large difference in our counts between years.

Bullfrog tadpoles were observed only in 2 ponds in late summer in 1997. One pond near the Gravel Pit was small and will likely dry up or freeze to the bottom during winter. The other pond, between Barn Slough and Mailbox Pond, was also used as a breeding site in 1996 (Hendricks 1997). Bullfrog tadpoles were not observed in North Slough either early or late in the summer in 1997, although some occupied that site in 1996 (Hendricks 1997). Bullfrog reproduction is undoubtedly occurring in a larger number of water bodies on the Refuge, and our lack of knowledge about the movements and microhabitat use by bullfrog tadpoles within any water body contributed to our poor sampling success. Use of minnow traps or some other funnel trap design (see Smith and Rettig 1996, Adams et al. 1997) probably would increase bullfrog tadpole detection.

Bullfrogs were the only amphibians observed on the Refuge during early (May-July) searches. However, one female and one male adult spotted frog (*Rana luteiventris*) were encountered during late-season surveys of the same water bodies (one at North Slough, one west of the residence). Spotted frogs were not detected on the Refuge during surveys in 1996, even though they are common in the Bitterroot Valley (Hendricks and Reichel 1996) and had been seen

at the nearby Florence Bridge Fishing Access. Breeding locations of spotted frogs on the Refuge, if any, are not currently known.

The rarity of spotted frog encounters, and lack of spotted frog tadpoles, suggests that they are relatively rare on the Refuge. Exclusion by bullfrogs may be occurring, as suitable habitat for spotted frogs is common on the refuge. However, spotted frogs breed in gravelly-bottomed ponds with weedy margins not dominated by cattails. The large ponds dominating the landscape at the Refuge, which are readily used by bullfrogs, are probably unsuitable for spotted frog reproduction. Timing of reproductive activities might also minimize bullfrog interference with spotted frog breeding. Some spotted frogs breed in early spring; egg masses and small tadpoles have been seen in mid-May (Hendricks and Reichel 1996). Bullfrogs breed in mid-summer. However, bullfrogs are known to exclude other ranids through a variety of mechanisms, including competition for food by bullfrog tadpoles, and predation by adult bullfrogs on smaller tadpoles and adult frogs. Bullfrog habitat use and inter-specific interactions with spotted frogs should be examined further.

Painted Turtle Surveys--Painted turtles occurred throughout the refuge (Figure 2). We observed an average of 7.3 turtles per pond (range 0-60). No turtles were observed in Ponds 3, 4 and 6, but they may occur here at other times. Early in the summer, turtles were not observed in Pond 1, but occurred at that time in nearby Pond 2. Late in the summer, when Pond 2 was almost dry, turtles were observed in Pond 1 but not in Pond 2. A similar late-summer distribution of turtles for these two ponds was observed in 1996 (Hendricks 1997). This suggests that turtles are moving between ponds as drying occurs, as documented in other studies (e.g., Zweifel 1989).

Figure 2. Painted turtle sightings (circles) and turtle nests (triangles) on Lee Metcald NWR, summer 1997 (unavailable)

We observed an average of 13 juvenile turtles (range=8-16 juveniles) approximately 5 cm in carapace length basking on algae mats in Ponds 13, 10, and 2. Juvenile turtles probably occur in other ponds where adult turtles are abundant, such as Ponds 5, 8 and 12. We also found small adult turtles (average carapace length ~ 10 cm) among basking turtles on logs in Ponds 5, 12, 13, and Francios Slough. Average size distribution of adult basking turtles was skewed toward the large (average carapace length ~20 cm) size class, with over 70 % of turtles classed as large. Small and medium (average carapace length ~15 cm) turtles averaged 13 % and 14 % of all turtles observed basking on logs. The large size of large adult painted turtles is striking (S. Corn, USGS BRD, pers. comm.) However, because painted turtles are long-lived, the high proportion of adult turtles in the largest size class is not unusual.

Other General Survey Results--Other reptiles seen on the Refuge included one western terrestrial garter snake (*Thamnophis elegans*) near Pond 2, and one common garter snake (*T. sirtalis*) near Pond 12. We did not observe long-toed salamanders (*Ambystoma macrodactylum*) on the refuge, but they are known to occur in some locations (P. Gonzales, pers. comm.). As previously mentioned, two spotted frogs were encountered (one near North Slough and one west of the residence area).

Painted Turtle Nests--Painted turtle nest habitat (usually sparsely vegetated gravel or sand near water) appeared to be more available on the north part of the Refuge, where the majority of turtle nests were found. However, turtle nests were also found at Barn Slough and the east side of Pond 5, and probably occur around Francios Slough and Pond 2 (turtles were observed at these

last two sites). The largest nesting areas appear to be on the slopes and sparsely vegetated upland NE of Pond 13, the bank along the E side of Pond 12, the railroad tracks surrounded by Pond 10, North Slough, Pond 11, and Otter Pond. Depredated turtle nests were observed more frequently north of Pond 4. Additional nesting areas are indicated in Figure 2. Many of the sandy banks in the southern part of the refuge appear to be too heavily vegetated for use by nesting turtles. A few depredated nests were found in the center of roads, suggesting that turtle nesting activity may be restricted to gravel roads in this portion of the Refuge.

Predation on painted turtle nests was high (Table 1), although our sample of initially undisturbed nests was small and may not be representative of nest fate for the entire Refuge population. Three of 11 nesting females did not complete their nests (i.e. nests were abandoned before eggs were laid by the female turtle), perhaps because we disturbed them or possibly because the site chosen for excavation proved unsuitable (see Christens and Bider 1987). Two (25 %) of 8 completed nests remained intact as of September 11, 75 and 78 days after egg laying. Six (75%) of 8 completed nests were destroyed by predators after an average of 24-31 days (SE=32.0-35.1, range=1-77 days; Table 1). The rate of nest predation we observed was higher than that reported in other studies of this species (see Tinkle et al. 1981, Snow 1982, Christens and Bider 1987, Lindeman 1991), where nest loss to predators ranged from 0-43.8% (sample size of completed nests ranged from 13-81). An additional 12-25% nest loss was due to other causes (hatch failure, flooding, etc.). In a study of three nesting turtle species (not including painted turtle) Temple (1987) noted predators destroyed 63.6% of 22 nests. It is reasonable to conclude from available information that nest failure in many painted turtle populations probably exceeds 50%,

Table 1. Persistence of new painted turtle nests on Lee Metcalf NWR during summer 1997. Last nest check was 11 September.

Nest no.	Date established	Date depredated	Days undisturbed[1]
1	June 21	September 4	60-75
2	June 25	-	at least 78
3	June 25	false start	-
4	June 25	July 1	3-6
5	June 26	September 11	70-77
6	June 28	false start	-
7	June 28	-	at least 75
8	July 3	July 28	9-25
9	July 3	July 7	0-4
10	June 29	false start	-
11	July 3	July 7	0-4

[1]Note: a range of dates indicates the shortest and longest possible interval due to the time between the last date when nest was observed intact and the date when predation was noted.

but that predation on Refuge turtle nests may be exceptionally high even for painted turtles. The need for a more conclusive study of painted turtle nest success on the Refuge is indicated.

Predation of painted turtle nests on Lee Metcalf NWR was likely due to vertebrate predators, based on tracks and the appearance of eggshell fragments we found. Skunk and canid tracks were present at and around nests. Potential predators on turtle nests that we observed on the Refuge at other times included striped skunk (*Mephitis mephitis*) , raccoon (*Procyon lotor*), and coyote (*Canis latrans*); each of these species has been identified previously as a predator of turtle nests (see Snow 1982, Christens and Bider 1987, Temple 1987). Refuge personnel reported that Common Ravens (*Corvus corax*) may destroy some nests.

Bullfrog and Bass Diets--Bullfrogs on the Refuge consumed mostly invertebrates (Tables 2 and 3). Dragonflies and damselflies were the most common items found in the bullfrog stomachs (n = 21) we examined. No turtles were found in the frog stomachs, and only one vertebrate, a small unidentified fish, was present. Other studies of bullfrog diet report similar results. Bullfrogs occasionally eat turtles (e.g., Korschgen and Moyle 1955, Korschgen and Baskett 1963, McKamie and Heidt 1974), but no study we are familiar with has reported large numbers in bullfrog diets. Although our sample sizes were small, it is reasonable to conclude that predation on hatchling turtles is uncommon.

Largemouth bass on the refuge ate a variety invertebrates (e.g., insects and snails) and small fish (Table 4). Because of the relatively large sample of bass collected (n = 37), we can reasonably conclude that bass do not commonly eat turtles in mid-summer (the time of our sampling).

Table 2. Bullfrog diets (number of stomachs present, proportion of non-empty stomachs with taxon, and average number of individuals per stomach) in early summer in different ponds on the Lee Metcalf National Wildlife Refuge. Sample size (n) is number of stomachs examined.

Pond No.	Taxon	Number of stomachs	Proportion of stomachs	Average number per frog
10	Odonata: Zygoptera (adult)	3	0.50	9.7
(n=6)	Arachnida (spiderling)	1	0.17	6.0
	Hymenoptera (nymph)	1	0.17	2.0
	Orthoptera: Acrididae	1	0.17	2.0
	Hirudinea	1	0.17	1.0
	Arachnida	1	0.17	1.0
	Odonata: Anisoptera (nymph)	1	0.17	1.0
	Coleoptera: Dytiscidae (adult)	1	0.17	1.0
	Insecta	1	0.17	1.0
	unknown worm	1	0.17	1.0
13	Odonata: Zygoptera (adult)	10	0.77	5.4
(n=13)	Coleoptera (adult)	3	0.23	2.3
	Odonata: Anisoptera (nymph)	3	0.23	1.7
	algae/empty	3	0.23	-
	Odonata: Anisoptera (adult)	2	0.15	3.5
	Coleoptera: Gyrinidae (adult)	2	0.15	2.0
	Gastropoda	1	0.08	2.0
	Hemiptera: Nepidae	1	0.08	1.0
	Hymenoptera: Formicidae	1	0.08	1.0
	Arachnida	1	0.08	1.0
	unidentified fish	1	0.08	1.0

Table 3. Some prey items taken by bullfrogs in late summer in different ponds on the Lee Metcalf National Wildlife Refuge. (n=1 frog from each pond).

Pond	Taxon	Number of prey items
North Slough	Orthoptera: Acrididae (adult)	1
	Diptera: Ptychopteridae (larva)	1
	Unidentified insect	1
13	Odonata: Anisoptera (adult)	4

Table 4. Some prey items taken by large-mouth bass in summer in different ponds on the Lee Metcalf National Wildlife Refuge. Sample size (n) is number of stomachs examined.

Pond	Taxon	Number of occurrences
8	Gastropoda	4
(n=14)	bass/fish	3
	Odonata: Zygoptera	2
	Hemiptera (adult)	1
	Annelida	1
	empty	5
10	Hemiptera: Corixidae	7
(n=23)	bass/fish	5
	empty	4
	Odonata: Zygoptera	4
	Odonata: Anisoptera	2
	Gastropoda	2
	Orthoptera	1
	Arachnida	1
	unidentified insects	6
	other unidentified invertebrates	5

Conclusions--The observation of juvenile and small adult turtles suggests that population recruitment has occurred in the last few years. Painted turtle nest predation appears to be high on the Refuge, but our sample of nests for determining nest fates was small. Because of small sample size, it is difficult to assess the significance of the observed predation rate. Hatchling turtles are not common in the diets of bass or bullfrogs (we failed to detect any). They may occur in the diet with greater frequency than our data indicate, but it is not likely that recruitment of young turtles into the Refuge population is significantly affected by either bass or bullfrogs.

POTENTIAL DIRECTIONS FOR FUTURE RESEARCH

Our work in 1997 documented the continued use of the refuge by spotted frogs, although their occurrence is not surprising. The rarity of spotted frogs on the refuge is striking, however. Competitive exclusion on spotted frogs by bullfrogs may be occurring. Future research should focus on bullfrog interaction with native amphibians on the refuge. Microhabitat characteristics of areas used and not used by bullfrogs should be contrasted to determine abiotic and biotic conditions that may favor (or exclude) bullfrogs. The presence of spotted frogs in areas adjacent to the refuge, and their continued reappearance on the refuge, suggests that this species would rapidly recolonize the refuge under the right conditions. Proper conditions probably includes the reduction or elimination of bullfrogs and, perhaps, large-mouth bass.

The location of bullfrog breeding sites on the refuge remains an open question. We know that breeding bullfrogs occur in a number of ponds on the refuge, but observations of bullfrog tadpoles are rare. The distribution and abundance of bullfrog tadpoles needs to be examined

further, perhaps by using minnow or funnel traps in ponds of all sizes. Searches should also be conducted for tadpoles of other amphibian species. Again, the microhabitat characteristics of areas of occurrence (or exclusion) of different species should be described. This information could be used to determine the range of conditions that favor bullfrogs, from which predictions could be made regarding their potential range in Montana. Likewise, conditions that exclude them could be used to enhance habitat for native amphibian species.

Painted turtles appear to be persisting on the Refuge. The range of body sizes indicates that recruitment continues to occur. However, it remains to be determined if recruitment is sufficient to maintain a stable population. Further work with this species to determine the details of the population structure and immigration rates onto the refuge from surrounding areas would require a large commitment of time and money that may not be warranted at this time. However, if the Refuge is interested in pursuing research on painted turtles, the following points need addressing:

1) Turtle nesting must have peaked before we began nest searches; nest searches should start earlier next year (late May-early June). This is early relative to what is expect at this latitude.

2) Predation by bullfrogs on hatchling turtles may not be common because hatchlings first enter the water before bullfrogs are active, and probably grow and find refugia before bullfrogs big enough to eat small turtles are eating voraciously (after breeding ends, in mid-July).

3) Bass may eat turtle hatchlings in spring; a spring sample should be collected.

4) Mark hatchling turtles at emergence. Conduct long-term marking and monitoring of all age classes of turtles.

5) Identify ways of protecting turtle nests from predators, if it is concluded that reduction in nest predation is necessary. A comprehensive study of painted turtle nest fates seems a necessity, with sample sizes much larger than those presented in this report.

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